# Chapter 3
# Implications for vascular plants

## 3.1. Introduction

In the laboratory setting, some plant species will absorb perchlorate when exposed to perchlorate via irrigation water. This has been explored for possible phytoremediation (Nzengung, 1999; 2000). Some investigators have speculated that bacteria are responsible for perchlorate reduction in plants. Perchlorate-reducing monera have been identified by several laboratories, and cultured from a variety of sources (including Las Vegas Wash sediments, food processing sludge, and sewage sludge) (Logan, 1998; Coates, 1999; Coates, 2000). This suggests that perchlorate-reducing bacteria are active in the environment. On the other hand, the bacteria isolated thus far prefer oxygen over nitrate over perchlorate. In order for perchlorate reduction to occur, the water must be anoxic, and all of the nitrate must have been consumed. Moreover, these bacterial cultures require a suitably moist environment. Arid soils or regions with low rainfall may not sustain their growth. Natural attenuation probably varies around the nation, depending on local factors; therefore, it is not possible to draw any conclusions about the ecological impact of using fertilizers that contain perchlorate, for they may be applied in areas where bacterial degradation occurs.

Due to the reported occurrence of perchlorate in certain water resources and in certain fertilizer products, several groups have begun to address the extent and significance of perchlorate uptake by plants. For example, if produce is grown using perchlorate-tainted irrigation water or fertilizers and the perchlorate is retained in the edible portions, this might constitute a route of human exposure. The possibility of exposure would be further increased if perchlorate were shown to survive various types of processing. Unfortunately, experimental results that definitively gauge the extent of risk from this route of exposure have not yet been published. However, some significant progress toward this goal has been made. Work is ongoing in this area, and several projects are proceeding at the Institute of Environmental and Human Health of Texas Tech University.

## 3.2. Complicating factors

One problem with uptake studies is the possibility of convolved influences on uptake. There are perchlorate absorption data available for only a few species of vascular plants. The absorption and accumulation of anionic solutes can be affected by many physical and chemical properties, such as concentration, size, charge density, and aquation.

### 3.2.1. Chemical influences on ion transport

Ion transport through plasma membranes occurs via transport proteins (Raven, 1999). At present, there are no published reports on the transmembrane transport of perchlorate in plants at the molecular or cellular level. In the absence of studies specifically examining influences on transmembrane perchlorate transport, it is necessary to consider whether previously published or ongoing uptake studies have accounted for likely influences.

Pertechnetate ($TcO_4^-$) is similar in size (ionic radius) and Gibbs free energy of aquation (Moyer, 1997). In aerobic aquatic environments, pertechnetate is highly mobile, accumulated by plants, and not appreciably retained by many soils (Sheppard, 1991); therefore, it is like perchlorate in many respects. One respect in which it differs is its ease of reduction to an immobile species, $TcO_2$ (Tagami, 1996). Unlike perchlorate, many papers have been published on the fate and transport of pertechnetate in food plants due to its release after use in medical imaging or from nuclear installations. Given the similarities between $ClO_4^-$ and $TcO_4^-$, those factors that influence pertechnetate uptake should be either accounted for or ruled out in perchlorate uptake studies. Depending on the plant, pertechnetate absorption and accumulation can be affected by nitrate (Kriger, 2000; Echevarria, 1996; 1998), sulfate (Cataldo, 1978; 1983), and phosphate (Cataldo, 1978; 1983; Echevarria, 1996; 1998). No studies on perchlorate uptake have attempted to account for the impacts of other ionic species.

### 3.2.2 Concentration

Active transport of perchlorate has not been reported. However, both active and passive transport of an ion are affected by its concentration (Raven, 1999). All ions experience an electrochemical gradient which is determined in part by the ion's concentration. Past and ongoing studies have exposed plants to much higher concentrations than are present in the environment.

For example, Hutchinson and coworkers are presently studying greenhouse-grown lettuce irrigated with perchlorate-tainted water. Lettuce is of particular importance for assessing the risk of perchlorate to the food supply since much of the lettuce produced in the U.S. is irrigated with perchlorate-tainted water. Also, lettuce has a high water content and virtually the entire aboveground plant is consumed without cooking or processing. Lettuce plants are watered with one of five different concentrations of perchlorate (0.1, 0.5, 1.0, 5.0, and 10.0 μg $mL^{-1}$) for a period of 90 days following planting. At various intervals of time, whole plants were harvested and divided into green tissue and root samples; each sample was analyzed for perchlorate. The analytical method was adapted from Ellington and Evans (2000). Levels of perchlorate rise steadily over the first 50–60 days, and then generally level off. The amount of perchlorate detected in the leaves correlates with dose. As an example, at about 50 days into the experiment, the lettuce irrigated with 10.0 $\mu g\ mL^{-1}$ perchlorate exhibits a perchlorate content of about 3 $mg\ g^{-1}$ on a lettuce dry matter basis. Since lettuce is about 90% water, this would amount to about 0.30 $mg\ g^{-1}$ on a wet mass basis. Experiments are underway to determine whether lettuce has the capability to degrade perchlorate if the supply of the contaminant is stopped; however, initial data suggest that increases in biomass are responsible for the apparent reduction in perchlorate content. Therefore, a decline in concentration (e.g., expressed as $mg\ g^{-1}$) does not adequately reflect the situation. While the preliminary results from these studies (Hutchinson, 2000) represent progress in understanding perchlorate in consumable produce, it must remembered that the perchlorate concentrations are about 500 times the concentrations in perchlorate-tainted irrigation water and that results obtained under laboratory growing conditions cannot be directly extrapolated to edible agricultural produce.

### 3.2.3. Tissue-specific accumulation

All studies of perchlorate occurrence in plants have focused on the leaves, stalks, and wood. While the leaves of some plants (e.g., lettuce) are consumed, the leaves of many others are not. Other than lettuce, no studies have been conducted on absorption and accumulation in edible fruits and vegetables. Therefore, information on uptake by *Tamarix ramosissima* (Urbansky, 2000C), *Salix* (Nzengung, 1999; 2000) tobacco (Wolfe, 1999) and *Myriophyllum aquaticum* (Susarla, 1999B; 1999C) cannot be directly applied or extrapolated to food crops. While many ions are transported via the xylem, the phloem supplies many of the nutrients to fruit. Although dissolved salts are carried into the xylem along with the water, the phloem relies on active transport. Because the edible portions of many plants (e.g., tubers, roots, some fruits) experience little to no transpiration, the phloem—rather than the xylem—carries both inorganic and organic materials for nutrition (Salisbury, 1992). For example, nitrate is almost never present in phloem (Salisbury, 1992), and is rarely found in fruit.

It is worth pointing out that studies on pertechnetate uptake showed that this anion was selectively accumulated in specific parts of plants and generally not in the consumable tissues. Cataldo (1986), Echevarria (1997), and Gast (1978) demonstrated that a variety of food plants could absorb and accumulate pertechnetate, with most of the ion in the roots or leaves rather than edible portions. While $TcO_4^-$ was shown to enter tomato leaf tissue via the xylem (Krijger, 1999), the fruit—which is actually consumed—was never tested. Accordingly, these observations demonstrate the importance of sampling consumable plant tissues and not just foliage. Furthermore, they warn against drawing inferences about fruit (or other edible portions) based on occurrence in foliage.

### 3.2.4. Soil sorption

It is generally accepted that perchlorate adsorbs to soil particles through outer-sphere complexes where the ions engage in simple electrovalent bonds and serve to balance electric charge on the surface (Sparks, 1995; Sposito, 1989). Such adsorption is often influenced by pH due to protonation of mineral oxo moieties, as is the case with goethite (Gurol, 2000; Sasaki, 1983) or $\gamma$-$Al_2O_3$ (Sasaki, 1983). Similar outer-sphere behavior has been observed by others as well, especially when perchlorate salts have been used to vary ionic strength and to provide a competitor to probe adsorption of another anion (Bourg, 1978; Gisler, 1980; Hundal, 1994; Ji, 1992; Kummert, 1980; Rhue, 1990; Sadusky, 1991; Sigg, 1981; Zachara, 1988; Zhang, 1996). The number of available binding/exchange sites is finite, but very large, permitting soil to act as a reservoir for anions. Outer-sphere complex formation is reversible and generally labile, but the sheer number of active sites can lead to a buffering effect on aqueous ion concentration, especially if the soil is far from saturated with the ion. This phenomenon is further affected by ionic strength. Under high ionic strength conditions (i.e., large concentrations of soluble and dissociable salts), less adsorption is possible for any one particular ion. In the case of diffuse ion swarms, where aquated counterions (anions in this case) hover near the surface in an ion-pair like situation, association with the positively charged surface is fleeting and constantly changing, with no real effect on availability of the ion to the aqueous phase.

Even though soil scientists routinely use perchlorate salts as indifferent electrolytes, it is possible that some soil types could resist desorption. In addition, sorption phenomena in soil are often more complicated than those in simpler (e.g., aqueous) chemical systems. Despite the principle of microscopic reversibility, which requires that the same pathway for adsorption be available for desorption, the thermodynamics of the sorption equilibrium affect the activation energy of the desorption process. Consequently, it is not unusual for desorption to occur via a different mechanism than the simple reverse of the one for adsorption (Sparks, 1995). Sorption phenomena have not been accounted for in any of the adsorption-accumulation studies conducted thus far. Transport of ions through soil is also a complex process (Jury, 1992; Goldberg, 1992; Selim, 1992). It can be difficult to account for in the laboratory setting due to the additional impacts of hydrologic, meteorologic, and geomorphologic factors, which are location-dependent. Moreover, ion exchange is often transport-controlled (Sposito, 1994).

### 3.2.5. Summary

To be applicable, studies of perchlorate absorption and accumulation must control for a variety of complicating factors. The presence of other anions either from co-administration in fertilizers or background salts present in the water supply may suppress uptake. Interspecies variation in absorption mechanisms may lead to differing levels of absorption and differing locations of accumulation. It is important to know if accumulation occurs in fruits versus in leaves or as a result of foliar application versus root application (as in irrigation).The rate of harvesting may lead to different rates of uptake by disrupting normal physiological processes in the plants. Lastly, the effect of soil (primarily sorptive in nature) must be considered. Careful agronomic studies are required to account for such influences, which are likely to complicate studies on the impact of contaminated irrigation water, too.

## 3.3. Difficulties in analysis

Another problem that has delayed accurate and definitive studies of perchlorate uptake by edible plants is the difficulty of analyzing for perchlorate in plant materials. Ion chromatography is currently the recommended method for routine analysis of inorganic ions such as perchlorate. It is a sensitive, reliable, and easily-implemented technique when perchlorate occurs in a matrix that has a relatively low level of total dissolved solids (TDS). Unfortunately, in a matrix with high TDS (which largely equates to high ionic strength), other ions can swamp the conductivity detector and effectively mask the signal from perchlorate. This has proven to be a very difficult problem with extracts of plant materials, many of which exhibit high TDS. In addition to potentially high levels of other inorganic ions, extracts of plant materials typically contain amino acids, other carboxylates or carboxylic acids (e.g., citrate, ascorbate, fatty acids) sugars, and nucleotides; all of which contribute to the ionic environment of the sample (Ellington, 2000). Ion chromatography is not alone in this regard. Other techniques and methods suitable for reasonably dilute drinking water matrices (Urbansky, 2000C; Magnuson, 2000A; Magnuson, 2000B) cannot be readily applied to fertilizers or botanical and physiological fluids. The problems of trace ionic analysis have led to development of other methods that rely on instrumentation that is common in high-end research laboratories, such as asymmetric waveform ion mobility mass spectrometry (Handy, 2000; Ells, 2000) or tandem MS-MS systems (Koester, 2000). Another option used for eliminating dissolved matter is selective preconcentration of the perchlorate on an anion-exchange resin (personal communication from Baohua Gu).

Recently, Ellington and Evans (2000) have reported an IC-based method for the analysis of perchlorate in plant materials, which greatly reduces interferences from high TDS. Their method involves first freeze-drying the plant material and then grinding it through a 30-mesh screen. The ground material is then mixed with water and heated for 0.5 hours in a boiling water bath in order to saturate the dry material and to precipitate proteins. The saturated samples are then shaken and stored at 3°C overnight. Next, the samples are centrifuged and the supernatant is filtered. With most plant materials, this produces a very highly colored solution that contains numerous inorganic and organic ions. Prior to analysis, Ellington and Evans add these highly colored extracts to alumina sorbent (DD-6) and allow them to stand for 20 hours at 3 °C. Finally, the extracts are filtered through an activated cartridge that contains divinylbenzene. The highly colored extracts are rendered colorless, and the ionic level is reduced dramatically. The minimum reporting level (MRL) of perchlorate in lettuce and tomato was found to be approximately 250 $\mu g\ g^{-1}$ on a wet mass basis.

Lettuce and tomato were chosen as representative plants because they are considered high priority candidates for screening studies of perchlorate in foodstuffs (Ellington, 2000) However, it should be noted that in this work, native perchlorate was not detected in any produce, nor was the method applied to any edible plants that were grown with intentional exposure to perchlorate. Instead, perchlorate was spiked into the extraction water for one half of the duplicate freeze-dried samples, while one half were extracted with pure water; consequently, sorptive loss to plant tissue is not entirely precluded. Ellington and Evans (2000) were able to evaluate the efficacy of the clean-up procedure for lettuce and tomato in light of the impact on the known perchlorate concentration in the spiked extracts. They observed no loss of perchlorate in spite of the efficient removal of other inorganic (and also organic) ions. This is tentatively attributed to preferential and competitive adsorption on the alumina. This assertion is supported by the observation that 40% of perchlorate is lost from solution when exposed to alumina in the absence of other ions. However, loss of perchlorate is not expected when the method is applied to plant material because most extracts have high levels of TDS. Despite some remaining uncertainties, the method of Ellington and Evans (2000) advances the assessment of potential risk posed by eating produce grown in the presence of perchlorate-tainted irrigation water or fertilizer.

## 3.4. Implications of perchlorate absorption and accumulation

An obvious concern raised by finding measurable perchlorate concentrations in plant tissues is whether this ion can affect food crops. Most domestic crops are fertilized using commodity chemicals with no known link to perchlorate contamination. Some crops (e.g., corn, wheat, and rice) are fertilized with nitrogen fertilizers that should be perchlorate-free because of the manufacturing processes. There is no reason to suspect any perchlorate associated with growing grains.

The only crops with documented use of Chilean nitrate products are tobacco and citrus fruits. Data on application of perchlorate-containing fertilizers is sparse or nonexistent, and it is not possible to estimate the ecological impact in any meaningful way. Modest information is available on uptake by tobacco, but this is not a food crop, and the use of Chilean nitrate salts appears to be locale-dependent.

Depending on the season, sources of fresh and processed fruits and vegetables vary considerably between domestic and imported. EPA has no data regarding the reliance on perchlorate-containing fertilizers for food crops grown outside the U.S. It is not known whether fruits or vegetables absorb and retain perchlorate ion under typical growing conditions (Cf. §3.2); accordingly, it is not possible to say whether produce can serve as an exposure route at this time. There are no published data on perchlorate in imported produce, no published data on perchlorate in domestic produce, and minimal data from controlled laboratory or field experiments on absorption and accumulation in food crops; therefore, it is impossible to assess whether foodstuffs contribute to perchlorate consumption in humans or whether drinking water provides the entire body burden.

Even if many food plants can be shown to absorb and retain perchlorate under some conditions, the primary source of this contaminant is irrigation water. However, merely exposing the growing plant to perchlorate does not imply absorption or accumulation in the edible portions. In the studies on uptake by lettuce seedlings, the plants have been subjected to perchlorate concentrations many times higher than those encountered in irrigation water. In addition, the confounding factors described previously and the harvest conditions have not been accounted for.

Because aerospace industries, perchlorate manufacturers, and military bases that use perchlorate salts are fairly localized geographically, most of the country's agricultural products should be free from exposure via tainted irrigation water. On the other hand, some produce is largely supplied by regions that irrigate with Colorado River water, which is known to contain perchlorate. Therefore, such produce represents a *potential* exposure route for consumers. There are currently no investigations underway to examine food crops with documented exposure to perchlorate via irrigation or fertilization. In the meantime, the gathering of data by fertilizer manufacturers who choose to screen their finished products as part of routine quality control measures to prevent any recurrence of perchlorate contamination is welcome. Some state or local authorities (e.g., agriculture departments or state chemists' offices) may choose to conduct periodic screenings in the course of their normal operations of assaying fertilizers to validate the grade. Unfortunately, it is impossible to be more specific since the source of the contamination previously observed has never been identified, but appears to have reflected an episodic, if not isolated, incident. There is inadequate evidence to suggest widespread or long-term perchlorate contamination in fertilizers used for the bulk of production farming operations nationwide. In fact, most evidence points to the contrary, but a modest level of continued vigilance would not be inappropriate. While the likelihood of exposure via agricultural sources is small due to the low consumption of Chilean nitrate salts and the low perchlorate concentrations therein, the significance of whatever exposure does occur is unknown in terms of food plant uptake or ecological impact.